Evaluation of Haematological Alterations in Children Infected by \textit{Plasmodium falciparum} Species in Enugu, Enugu State, Nigeria

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Authors’ contributions

This work was carried out in collaboration among all authors. Author LNO and SAU designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author EIO managed the analyses of the study. Author COO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Malaria accounts for a considerable amount of Morbidity and mortality with children bearing the greatest burden. The study aimed to investigate the haematological alterations in children infected by \textit{Plasmodium falciparum} (\textit{P. falciparum}) species. A case control study with a total of ninety-five microscopically confirmed \textit{P. falciparum} malaria infected children and fifty apparently healthy age and gender matched controls from Enugu State University Teaching Hospital, Parklane, Wesley Specialist Hospital and Akpugo Community Health Centre, Enugu were recruited for the study. Haematologic parameters were estimated using five part differential automated analyzer (Mindray).
BC 5300) with ethylene-diamine-tetra-acetic-acid anticoagulated blood. The result of *P. falciparum* infected male children revealed significant decrease (p < 0.05) in haematocrit (32.76 ± 4.82)%), haemoglobin (10.34 ± 1.46) g/dl, white blood cell (3.65 ± 2.81)x10^9/L, neutrophil (20.41 ± 15.16)% and platelet (228.41 ± 113.51) x10^9/L, compared to the controls; haematocrit (35.60 ± 2.70)%), haemoglobin (11.27 ± 0.90) g/dl; white blood cell (7.97 ± 2.55)x10^9/L, neutrophil (30.33 ± 8.04)% and platelet (315.45 ± 53.64) x 10^9/L. It also showed a significant increase (p < 0.05) in monocyte (3.28 ± 2.63)%), basophil (1.68 ± 1.15)% compared to the controls; monocyte (1.89 ± 1.10)%), basophil (0.44 ± 0.46)%. The results of *P. falciparum* infected female children revealed significant decrease (p < 0.05) in white blood cell (4.93 ± 2.95)x10^9/L, neutrophil (19.61 ± 15.14)%), mean corpuscular haemoglobin (23.58 ± 2.37)pg, mean corpuscular volume (74.67 ± 8.00)fl, and platelet (257.00 ± 129.55) x10^9/L compared to the controls; white blood cell (6.70 ± 1.75) x10^9/L, neutrophil (26.91 ± 7.97)%), mean corpuscular haemoglobin (25.12 ± 1.96)pg, mean corpuscular volume (79.13 ± 5.37) fl and platelet (303.13 ± 54.02) x10^9/L, significantly increased (p < 0.05) monocyte (3.38 ± 2.78)%), basophil (2.19 ± 1.36) as compared to the controls; monocyte (1.85 ± 0.82)%), basophil (0.48 ± 0.45)%. However, *P. falciparum* infected male children showed to be more anaemic compared to the infected females. Indeed, *Plasmodium falciparum* malaria infection in children has great impact on the haematologic parameters, thus its investigations serves as competent measures of differential diagnosis.

Keywords: Haematological alterations; children; Plasmodium falciparum; Enugu.

1. INTRODUCTION

Malaria remains a global burden with its complication in childhood posing a great threat to public health [1,2]. In hyperendemic and holoendemic malarial areas in Sub-Saharan Africa including Nigeria, it is the most widespread and life threatening human protozoal infection [3]. It is certainly one of the diseases exerting a huge economic burden on families, communities and the country at large [4,5]. While children under the age of five and pregnant women are particularly vulnerable, almost the entire population of Nigeria is at risk of contracting malaria [6].

The morphological and biochemical alterations on the red cell membrane induced by malaria infection is necessary for the survival of the parasite, its growth, cell differentiation, compartmentalization and nutrient uptake [6]. “The entrance of *P. falciparum* into red blood cells causes marked increase in inflammatory cytokine secretions (TNFα, IL-1, IL-10 and IFNγ), activation of endothelial cell (due to over expression of cell adhesion molecules; (ICAM-I VCAM-1), coagulation cascade activation (due to platelet consumption and endothelial damage), and parasitized red blood cells sequestration” [7].

Malaria is primarily caused by parasites transmitted from one person to another through the bites of infected female anophelines mosquitoes [8]. The protozoan parasites of genus *plasmodium* that infect humans include; *Plasmodium falciparum, Plasmodium ovale, Plasmodium vivax and Plasmodium malariae* with *P. falciparum* causing more deadly infection [9,10]. *Plasmodium falciparum* infections affect single and multiple organs resulting to organ failure, severe anaemia, cerebral malaria, coma and consequently death [11]. Other ways include; contact with infected blood (Faruk, 2016), from mother to foetus before/during delivery [12], congenitally acquired disease, sharing of contaminated needles and organ transplantation [13].

Haematological abnormalities are one of the most common complications in malaria as it involves the major cell lines [14]. Haematological changes associated with malaria include anaemia, leukocytosis/leucopenia, thrombocytopenia and disseminated intravascular coagulation [15]. The degree of these alterations differs with the degree of malaria endemcity, background haemoglobinopathy, nutritional status, environmental factors and malaria immunity [16]. Anaemia is one of the most common complications of malaria infection with the degree of anaemia correlating with parasitamia [17]. Its pathogenesis could be due to haemolysis of parasitized red blood cells, accelerated destruction of non-parasitized red blood cells, depressed and ineffective erythropoiesis, and increased splenic clearance [14].
change seen in *P. falciparum* infection [18]. It due to peripheral destruction or consumption by disseminated intravascular coagulation.

The prompt and accurate diagnosis of malaria is the key feature for its treatment/management and prevention of complication though it requires technical expertise and manpower [19]. Malaria is preventable and curable and increased efforts are dramatically reducing the malaria burden in many places [8].

The study was done to investigate the haematological alterations in children infected by *P. falciparum* species in Enugu, Enugu State, Nigeria.

### 2. MATERIALS AND METHODS

#### 2.1 Study Area

Enugu State was created on 27th August, 1991 and it is situated in the South-East geopolitical zone of Nigeria. It covers an area of 7,161km², located at 63°N of the equator and 73° of latitude. It has 17 local government areas with an estimated population of 3,269,837 in which 1,596,042 are males and 1,671,795 are females [20]. The state is predominantly occupied by Igbo’s mainly civil servants, students and business enterprise. The wet season is between the period of March and September when the breeding of anopheles mosquitoes and its bite are at peak. It shares borders with the following states; Abia and Imo to the South, Ebonyi to the East, Kogi to the North-West and Anambra to the West. Enugu State is the home of many polytechnics, colleges of education, universities including University of Nigeria, Nsukka. It has health facilities like University of Nigeria Teaching Hospital, Ituku-Ozalla, Enugu State University Teaching Hospital, Parklane and other government owned/private establishments around the State (Johnson, 2013).

#### 2.2 Subjects

A total of one hundred and forty five patients were studied. Ninety five were children infected with *P. falciparum* malaria between the ages of 1-10 years and fifty were apparently healthy children (controls), sex and age matched. The patients were not on antimalarial treatment as at the time of sample collection and had no associated infection on clinical examination.

#### 2.3 Study Design

This was a case control study focused on children between 1-10 years of age at Enugu State University Teaching Hospital, Parklane, Wesley Specialist Hospital, Enugu and Akpugo community Health Centre. This study ran from April, 2018 to September, 2018 covering the wet season when the rate of mosquito bite is at its peak. The patients were recruited consecutively and prospectively.

#### 2.4 Inclusion Criteria

Inclusive subjects were male and female children between the ages of 1-10 years who were not on antimalarial drugs. Only Patients positive for *P. falciparum* malaria infection were included in the study.

#### 2.5 Exclusion Criteria

Children who refused to give their consent were excluded from this study. Children with sickle cell disease trait were also excluded from this study because malaria parasite does not grow well in sickle cells.

#### 2.6 Sample Size

The sample size, n for the study will be calculated using the formula below (Araoye, 2004).

\[
n = \frac{Z^2(P)(1-P)}{d^2}
\]

Where,

- \(n\) = the desired sample size when the population is more than 10,000
- \(z\) = standard deviation usually set at 1.96, which corresponds to 95% confidence interval
- \(p\) = the proportion in the target population estimated to have a particular characteristics. (Prevalence and severity of malaria parasitaemia among children requiring emergency blood transfusion in tertiary hospital in Imo state, Nigeria) by [21] was 0.069
- \(d\) = degree of accuracy desired set at 5% (that is tolerated error of 5%) which is equal to 0.05.

Therefore, the minimum sample size,

\[
n = \frac{(1.96^2)(0.069)(1-0.069)}{0.05^2}
\]
\[ n = \frac{(3.8416 \times 0.069 \times 0.931)}{0.0025} = 99 \]

2.7 Sample Collection

Blood sample (2ml) was collected by venepuncture under asceptic conditions and the blood sample was transferred into the ethylene-diamine-tetra-acetic-acid (EDTA) container for malaria parasite test and haematological studies.

2.8 Parasitologic Examination of Blood Samples

Giemsa stained thick and thin blood films were prepared for each sample and parasitaemia evaluated per microliter of blood using the thick film preparation according to standard method described by World Health Organisation (Gilles, 1991) assuming a mean total leukocyte count of 8000/µl of blood. This was done using Olympus binocular microscope. A slide was considered negative when no malaria parasite was seen in 100 high power fields.

Parasitic density (mp/µl) = \( \frac{\text{Number of parasites} \times 8000/\mu l}{\text{Number of leukocytes}} \)

2.9 Laboratory Procedures

Haematological Analysis: Complete blood count was done using a five part differential Haematology autoanalyzer, Mindray BC-5300 (China).

2.10 Statistical Analysis

Data were subjected to descriptive statistics and analyzed using analysis of variance and student’s T-test. The probability value less than 0.05 were considered statistically significant.

3. RESULTS

3.1 Characteristics of Study Population

A total of one hundred and forty five children (symptomatic and asymptomatic) were sampled from hospitals in Enugu, Enugu State. It included 74 males (51%) and 71 females (49%) with male to female ratio of 1.04:1. The mean age of the infected males and females and that of the control group were similar (1-10 years) as shown in Table 1. All the infected patients were of \( P. falciparum \) malaria parasite specie and no patient with dual infection was included in this study.

3.2 Effect of Malaria on Haematologic Parameters between the Infected Male and Female Children with their Controls

Table 2 shows a comparison of haematologic parameters between \( P. falciparum \) infected male and female children with their controls. The \( P. falciparum \) infected males result revealed a significant decrease (\( p < 0.05 \)) in packed cell volume (PCV), haemoglobin (Hb), white blood cells (WBC), neutrophils, platelet and an increased (\( p < 0.05 \)) monocyte and basophil, as compared to the controls.

In \( P. falciparum \) infected females; WBC, neutrophil, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and platelet were significantly decreased (\( p < 0.05 \)) while monocyte and basophil were significantly increased (\( p < 0.05 \)) as compared to the controls. Other parameters did not differ significantly (\( p > 0.05 \)).

3.3 Effect of Malaria on Haematologic Parameters of the \( P. Falciparum \) Malaria Infected Children and Controls

A comparison of the haematologic parameters between the \( P. falciparum \) malaria infected children and the controls are shown in Table 3. The result revealed a significant decrease (\( p < 0.05 \)) in PCV, Hb, WBC, Neutrophil, MCH, MCV and a significant increased (\( p < 0.05 \)) monocyte, eosinophil and basophil in the infected children as compared to the controls. Other parameters did not differ significantly (\( p > 0.05 \)).

4. DISCUSSION

Alterations in haematological parameters in relation to malaria infections are common complications as it involves the major cell lines. The degree of these alterations differs with the rate of malaria endemicity, environmental factors, background haemoglobinopathies, immunity to malaria and nutritional status [14].

The observed significant decrease in the packed cell volume (PCV) of \( P. falciparum \) infected male children indicates that the males were slightly anaemic. Anaemia occurs when the Hb concentration falls below the normal for a person’s age, gender and environment. It is defined as Hb level < 11 mg/dl for both male and
female based on WHO cut-off value [17]. The pathogenesis of anaemia is obscure though it is thought to be caused by a combination of haemolysis of parasitized red cell, accelerated removal of both parasitized and un-parasitized red cells, ineffective erythropoiesis and splenic phagocytosis/pooling [22]. Normocytic Normochromic anaemia is mostly seen in

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Malaria infected patients</th>
<th>Controls</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>5.77 ± 2.63</td>
<td>5.74 ± 2.52</td>
<td></td>
</tr>
<tr>
<td>Parasite density (x10³ mp/µl)</td>
<td>2.08 ± 0.999</td>
<td>0.00 ± 0.00</td>
<td></td>
</tr>
<tr>
<td>Gender: Male</td>
<td>54</td>
<td>20</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>41</td>
<td>30</td>
</tr>
<tr>
<td>Total (n)</td>
<td>95</td>
<td>50</td>
<td>145</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Malaria infected male children</th>
<th>Controls</th>
<th>Malaria infected female children</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pcv (%)</td>
<td>32.76 ± 4.82</td>
<td>35.60 ± 2.70</td>
<td>32.03 ± 6.33</td>
<td>34.07 ± 2.28</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>10.34 ± 1.46</td>
<td>11.27 ± 0.90</td>
<td>10.11 ± 1.95</td>
<td>10.78 ± 0.77</td>
</tr>
<tr>
<td>WBC (x 10⁹/L)</td>
<td>3.65 ± 2.81</td>
<td>7.97 ± 2.55</td>
<td>4.93 ± 2.95</td>
<td>6.70 ± 1.75</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>20.41 ± 15.18</td>
<td>30.33 ± 8.04</td>
<td>19.61 ± 15.14</td>
<td>26.91 ± 7.97</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>72.19 ± 17.59</td>
<td>66.84 ± 7.86</td>
<td>71.90 ± 19.10</td>
<td>70.11 ± 7.67</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>3.28 ± 2.63</td>
<td>1.89 ± 1.10</td>
<td>3.38 ± 2.78</td>
<td>1.85 ± 0.82</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>0.93 ± 1.11</td>
<td>0.51 ± 0.50</td>
<td>0.92 ± 1.09</td>
<td>0.61 ± 0.52</td>
</tr>
<tr>
<td>Basophils (%)</td>
<td>1.68 ± 1.15</td>
<td>0.44 ± 0.46</td>
<td>2.19 ± 1.36</td>
<td>0.48 ± 0.45</td>
</tr>
<tr>
<td>RBC (x 10¹²/L)</td>
<td>4.34 ± 0.70</td>
<td>4.58 ± 0.45</td>
<td>4.33 ± 0.89</td>
<td>4.33 ± 0.47</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>75.10 ± 12.81</td>
<td>78.35 ± 4.33</td>
<td>74.67 ± 8.00</td>
<td>79.13 ± 5.37</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>24.13 ± 2.64</td>
<td>24.83 ± 1.25</td>
<td>23.58 ± 2.37</td>
<td>25.12 ± 1.96</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>36.65 ± 37.77</td>
<td>46.00 ± 64.32</td>
<td>31.62 ± 1.02</td>
<td>31.61 ± 0.74</td>
</tr>
<tr>
<td>Platelete (x 10³/L)</td>
<td>228.41 ± 113.51</td>
<td>315.45 ± 53.64</td>
<td>257.00 ± 129.55</td>
<td>303.13 ± 54.02</td>
</tr>
<tr>
<td>Parasitic density (x10³/mp/µl)</td>
<td>2.22 ± 1.09</td>
<td>0.00 ± 0.00</td>
<td>1.90 ± 0.84</td>
<td>0.00 ± 0.00</td>
</tr>
</tbody>
</table>

*Significance set at p < 0.05 (using T-tests and one way ANOVA)*

↓↑ denotes significant decrease and increase respectively as compared to the controls

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Malaria infected children</th>
<th>Controls</th>
<th>Sig. level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pcv (%)</td>
<td>32.45 ± 5.51</td>
<td>34.64 ± 2.55</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>10.24 ± 1.68</td>
<td>10.97 ± 0.85</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>WBC (x 10⁹/L)</td>
<td>4.20 ± 2.92</td>
<td>7.21 ± 2.17</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>20741 ± 15.09</td>
<td>28.27 ± 8.09</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>72.06 ± 18.16</td>
<td>68.80 ± 7.84</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>3.32 ± 2.68</td>
<td>1.86 ± 0.93</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>0.93 ± 1.10</td>
<td>0.57 ± 0.51</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>Basophils (%)</td>
<td>1.90 ± 1.26</td>
<td>0.47 ± 0.45</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>RBC (x 10¹²/L)</td>
<td>4.33 ± 0.78</td>
<td>4.43 ± 0.47</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>74.91 ± 10.95</td>
<td>78.82 ± 4.96</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>23.89 ± 2.53</td>
<td>25.00 ± 1.70</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>34.48 ± 28.48</td>
<td>37.36 ± 40.79</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>Platelete (x 10³/L)</td>
<td>240.75 ± 120.87</td>
<td>308.06 ± 53.66</td>
<td>p &lt; 0.05</td>
</tr>
</tbody>
</table>

*Significance set at p < 0.05 (using T-tests and one way ANOVA)*

↓↑ denotes significant decrease and increase respectively
*P. falciparum* infection. Okeke et al. (2014) reported a significantly lower male PCV as compared to the females in a study done in Anambra State. The findings of this study is in line with the research done by Kayode et al. [23] which reported a significantly lower PCV in malaria infected patients than the control. In another study, Ogbedo et al. [24] also reported a decreased PCV level in malaria infected patients in a study carried out in the rural communities in Eastern Nigeria.

The significant decrease in total white blood cell (WBC) count indicates leucopenia. Leucopenia is defined as total white blood cell count <4000/µl. This could be as a result of sequestration of leucocytes, accelerated destruction or decreased production. This finding is in accordance with the study by Okeke et al. [6] which reported a significantly lower WBC in infected patients compared to the controls. In another study, Kayode et al. [23] indicated significantly increased WBC in malaria and malaria typhoid co-infected patients. He attributed the higher WBC count to increased production of leucocytes at the onset of an infection to wade off the invading pathogen/parasite.

In a study conducted by Maina et al. [25] on Kenya children, significant increase in monocyte was recorded in person with malaria infection than in the control. The observed increase in basophil might probably be due to allergic reactions caused by the bite of the mosquito. Report shows that eosinophil, basophil and mast cell as components of innate immune response plays an important role in the pathogenesis of malaria through histamine release [26].

The level of Neutrophils in *P. falciparum* infected children was found to be significantly lower than that of the controls. This is in line with the report of Senthilkumara et al. [27] which noted a decreased neutrophil count in malaria infected individual as compared to the control. But in contrast to Maina et al. [25] increased neutrophil reported which may be due to activated neutrophil production or release from the marrow or suppressed peripheral removal. The significant decrease in mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) could possibly be due to microcytosis resulting from nutritional or iron deficiency anaemia [28]. This finding is in contrast with Manas et al. [17] significant higher MCV and MCH in malaria infected patients in comparison to non-malaria infected patients.

The observed significant decrease in platelet count of *P. falciparum* malaria infected children indicates thrombocytopenia. Thrombocytopenia is defined as platelet count <150,000/µl. The mechanism of thrombocytopenia in malaria has not been described but it could occur due to decreased thrombopoiesis, immune mechanism, enhanced splenic uptake/sequestration, peripheral destruction induced by *P. falciparum* and disseminated intravascular coagulation [18]. This result is in agreement with the study by Francis et al. [11] which reported a significantly lower platelet count in malaria infected patients than the controls. The report of lower platelet count and low PCV conforms to the study by Bhawna et al, [16] showing parasitaemia and haematological alterations in malaria. Thrombocytopenia is one of the most common complications in malaria infections. Maina et al. [25] further stated that children infected with *P. falciparum* malaria exhibit some changes in haematological parameters with low platelet count and haemoglobin concentration being the two most important predictors of malaria.

5. CONCLUSION

In conclusion, *P. falciparum* malaria infection demonstrated a significant impact on haematological parameters. *Plasmodium falciparum* malaria infected children exhibit important haematological changes with low PCV, Hb, WBC and platelet being the most important predictors of malaria infection.

CONSENT AND ETHICAL CONSIDERATION

Ethical clearance was obtained from the Health Research and Ethical committee of the University of Nigeria Teaching Hospital, Ituku-Ozalla, Enugu, Enugu State. Informed consent was obtained from the mothers and caregivers of the children before administration of questionnaire and collection of blood sample.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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